

U.S. Patent Application No. 10/774,076
Attorney Docket No. 161 US UT01

AMENDMENTS

In the specification:

Please replace the paragraph beginning on page 2, line 29, with the following:

The present invention is directed to neutralizing anti-AR antibodies having the disclosed amino acid sequences or binding characteristics, which are not disclosed in the above-referenced publications. The *in vivo* efficacy of the anti-AR antibodies for treating psoriasis and cancer are tested in the present invention. These antibodies will find use for inhibiting cancer cell growth, ~~would wound~~ healing, enhancing skin quality, or/and treating psoriasis in a subject. The present invention is also pursuant to methods of treating psoriasis or epidermal and pancreatic cancers with any antagonists of AR, preferably, anti-AR antibodies, and more preferably, the antibodies claimed herein.

Please replace the paragraph beginning on page 7, line 12, with the following:

Figure 2 depicts the panel of positive monoclonal antibodies generated against human AR. The depicted ~~dated~~ data includes the antibody isotypes, binding characteristics, and neutralizing capability.

Please replace the paragraph beginning on page 15, line 7, with the following:

“Conservatively modified variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical or associated, e.g., naturally contiguous, sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode most proteins. For instance, the codons GCA, GCC, GCG, and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to another of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes silent variations of the nucleic acid. One of skill will recognize that in certain contexts each codon in a nucleic

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acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, often silent variations of a nucleic acid which encodes a polypeptide [[is]] are implicit in a described sequence with respect to the expression product, but not with respect to actual probe sequences.

Please replace the paragraph beginning on page 16, line 5, with the following:

The term "AR" refers to nucleic acid and polypeptide polymorphic variants, alleles, mutants, and interspecies homologues of amphiregulin, including those that: (1) have a nucleotide sequence that has greater than about 60% nucleotide sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, or more preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater nucleotide sequence identity, preferably over a region of at least about 25, 50, 100, 200, 500, 1000, or more nucleotides, to a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:1; (2) bind to antibodies, e.g., polyclonal antibodies, raised against an immunogen comprising an amino acid sequence of SEQ ID NO: 1, and conservatively modified variants thereof; (3) specifically hybridize under stringent hybridization conditions to a nucleic acid sequence, or the complement thereof, encoding amino acid sequence of SEQ ID NO: 1 and conservatively modified variants thereof; or (4) have an amino acid sequence that has greater than about 60% amino acid sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200, or more amino acids, to an amino acid sequence of SEQ ID NO:1. An AR polynucleotide or polypeptide sequence is typically from a mammal including, but not limited to, primate, e.g., human; rodent, e.g., rat, mouse, hamster; cow, pig, horse, sheep, or other mammal. An "AR polypeptide" and a "AR polynucleotide," include both naturally occurring or recombinant forms.

Please replace the paragraph beginning on page 16, line 24, with the following:

A "full length" AR protein or nucleic acid refers to [[a]] an ovarian cancer polypeptide or polynucleotide sequence, or a variant thereof, that contains all of the elements normally contained in one or more naturally occurring, wild type AR polynucleotide or polypeptide sequences. For example, a full length AR nucleic acid will typically comprise all of the exons

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that encode for the full length, naturally occurring protein. The "full length" may be prior to, or after, various stages of post-translation processing or splicing, including alternative splicing.

Please replace the paragraph beginning on page 25, line 3, with the following:

The present invention provides for anti-AR antibodies comprising a mature heavy chain variable region comprising an amino acid sequence selected from SEQ ID NOs: 2-4 and 12 SEQ ID NOs: 2, 4, and 12. These antibodies may further comprise a mature light chain variable region comprising amino acid sequence selected from SEQ ID NOs: 3, 5 and 14. The present invention includes antibodies that bind to the same epitope of these antibodies. The determination of epitope type is accomplished by methods known in the art, such as a competition assay, which, for example, may be detected by changes in fluorescence intensity as measured by flow cytometry. In cases where the epitopes of the two antibodies are similar, the antigen-binding sites will be occupied by the first antibody and the second antibody conjugate will be unable to bind cells. This results in loss of signal of this conjugate, so that the fluorescence intensity will be reduced.

Please replace the paragraph beginning on page 25, line 14, with the following:

The present invention includes the analogs of the antibodies described herein. Preferred analogs include antibodies comprising heavy chain variable regions having about at least 60%, 80% or 90-95% amino acid sequence identity of SEQ ID NOs: 2-4 and 12 SEQ ID NOs: 2, 4, and 12 and/or comprising mature light chain variable regions having about at least 60%, 80% or 90-95% amino acid sequence identity of SEQ ID NOs: 3, 5 and 14.

Please replace the paragraph beginning on page 36, line 28, with the following:

The 293H and Cos-7 cell lines were grown according to the supplier's instructions (ATCC). Plasmid DNAs of AR expressing proteins were transfected into 293H cells using TransIT-293 (Fisher) transfection reagent according to the manufacturer's instructions for secreted proteins. Culture media were harvested 3-5 days later, and the expressed AR fusion proteins were purified by affinity column chromatography. Plasmid DNA for cell surface expression was transfected into Cos-7 cells using Lipofectamine 2000 Lipofectamine 2000®

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transfection formulation (Life Technologies). Cells were analyzed for surface expression and cloned.

Please replace the paragraph beginning on page 37, line 9, with the following:

Recombinant human AR was purchased from R&D Systems and used to immunize Balb/c mice via either the intraperitoneal [[of]] or footpad route. Briefly, mice were immunized intraperitoneally or in the hind footpads using 5-20 µg protein with an equal volume of Ribi adjuvant in a total final volume of 20 µl. Footpad immunizations were performed 4 times at 4 or 5-day intervals. Intraperitoneal immunization involved 4 immunizations at two-week intervals.

Please replace the paragraph beginning on page 40, line 1, with the following:

Another proliferation assay utilized HEKn (Human Epidermal Keratinocytes – neonatal) cells (Cascade Biologics). These cells proliferate to endogenously synthesized AR. HEKn cells were plated at 3×10^3 cells/well in 96 well black walled plates (Costar) on day 0. On day 1, wells were washed extensively with growth factor free medium (EpiLife Epilife[®], a liquid growth medium) (Cascade Biologics). PAR antibodies were then added at various concentrations (0.01 – 3 µg/ml). After 48 – 72 hrs, inhibition of endogenous amphiregulin induced proliferation was assessed by quantitation of ATP using a luminescent cell viability assay (CellTiter-Glo CellTiter-Glo[®]) (Promega Corp.).

Please replace the paragraph beginning on page 41, line 29, with the following:

As shown in Fig. 6, in the AsPC-1 prevention model, both PAR34 and PAR80 demonstrated efficacy in tumor volume reduction with no associated general associated general toxicity based on loss of body weight (data not shown).

Please replace the paragraph beginning on page 42, line 15, with the following:

Total RNA was extracted from approximately 10^7 hybridoma cells producing PAR34 using TRIzol reagent (Life Technologies, Inc., Rockville, MD) and poly (A)⁺ RNA was isolated with the PolyATract mRNA Isolation System (Promega Corporation, Madison, WI) according to the suppliers' protocols. Double-stranded cDNA was synthesized using the SMART RACE cDNA Amplification Kit (BD Biosciences Clontech, Palo Alto, CA) following the supplier's

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protocol. The variable region cDNAs for the heavy and light chains were amplified by polymerase chain reaction (PCR) using 3' primers that anneal respectively to the mouse gamma and kappa chain C regions, and a 5' universal primer provided in the SMART RACE cDNA Amplification Kit. For VH PCR, the 3' primer has the sequence 5'-GCCAGTGGATAGACTGATGG-3' (SEQ ID NO:6). For VL PCR, the 3' primer has the sequence 5'-GATGGATACAGTTGGTGCAGC-3' (SEQ ID NO:7). The VH and VL cDNAs were subcloned into the ~~pCR4Blunt TOPO~~ pCR4Blunt-TOPO® vector from a TOPO® cloning kit (Invitrogen Corporation, Carlsbad, CA) for sequence determination. DNA sequencing was carried out by PCR cycle sequencing reactions with fluorescent dideoxy chain terminators (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

Please replace the paragraph beginning on page 45, line 27, with the following:

One of the NS0 stable transfectants producing a high level of HuPAR34, clone v1#2, was adapted to and expanded in Protein Free Basal Medium-1 (PFBM-1) (Protein Design Labs, Inc.), expanded in PFBM-1 supplemented with Protein-Free Feed Medium-2 (PFFM-2) (Protein Design Labs, Inc.), and grown to exhaustion. After centrifugation and filtration, culture supernatant was loaded onto a protein-A Sephadex Sepharose® (chromatography medium) column. The column was washed with PBS before the antibody was eluted with 0.1 M glycine-HCl (pH 2.8), 0.1 M NaCl. After neutralization with 1 M Tris-HCl (pH 8), the eluted protein was dialyzed against PBS, 0.2 µm filtered, and stored at 4°C. Antibody concentration was determined by measuring absorbance at 280 nm (1 mg/ml = 1.4 A₂₈₀). Purified antibodies were characterized by SDS-PAGE analysis according to standard procedures. Analysis under non-reducing conditions indicated that HuPAR34 has a molecular weight of about 150-160 kD. Analysis under reducing conditions indicated that HuPAR34 is comprised of a heavy chain with a molecular weight of about 50 kD and a light chain with a molecular weight of about 25 kD. The purity of the antibody appeared to be more than 95%.